

AMENDMENTS TO THE SPECIFICATION

Page 1, before line 1, please insert

This application is a Division of U.S. Application Serial No. 09/920,954, filed on August 3, 2001, which is a Continuation of U.S. Application Serial No. 09/509,814, filed on April 6, 2000, now U.S. Patent No. 6,376,227, which is a 371 of PCT/JP98/04528, filed October 7, 1998.

Please replace the paragraph beginning on page 3, line 25 with the following:

Fig. 1 shows the effects of pH on the activity of alkaline protease KP43.

Fig. 2 shows the effects of pH on the stability of alkaline protease KP43 (40°C., 30 minutes).

Please replace the paragraph beginning on page 4, line 1 with the following:

Fig. 3 shows the effects of pH on the stability of alkaline protease KP43 (10°C, 24 hours).

Fig. 4 shows the effects of temperature on the activity of alkaline protease KP43.

Fig. 5 shows the effects of temperature on the stability of alkaline protease KP43.

Fig. 6 shows the effect of an oxidizing agent (50 mM hydrogen peroxide) on the activity of alkaline protease KP 43.

Fig. 7 shows N-terminal sequences of KP9860 protease and partially degraded products thereof (SEQ ID NOS:9-13, appearing in descending order in Fig. 7).

Fig. 8 shows primer sequences designed from an N-terminal sequence of KP9860 protease (SEQ ID NOS:9-13). 9860-N2, and its variants shown in Fig. 8, corresponds to SEQ ID NO:14. 9860-18k-RV, and its variants shown in Fig. 8, corresponds to SEQ ID NO:15. 9860-18k, and its variants shown in Fig. 8, corresponds to SEQ ID NO:16. 9860-25k-RV, and its variants shown in Fig. 8, corresponds to SEQ ID NO:17. 9860-25k, and its variants shown in Fig. 8, corresponds to SEQ ID NO:18. 9860-28k-RV, and its variants

shown in Fig. 8, corresponds to SEQ ID NO:19, 9860-28k, and its variants shown in Fig. 8,  
corresponds to SEQ ID NO:20.

Fig. 9 shows 57 bp PCR-amplified fragments and primer designs (primer 1 = SEQ ID  
NO:21, primer 2 = SEQ ID NO:22, primer 3 = SEQ ID NO:23, and primer 4 = SEQ ID  
NO:24).

Page 5, replace the last paragraph with the following paragraph:

The alkaline protease of the present invention preferably has an amino acid sequence shown by Sequence No. 1 or 2 in SEQ ID NOS: 1 or 2, or such a sequence in which one or more amino acids are deleted, substituted, or added. Sequence No. 1 SEQ ID NO: 1 differs from Sequence No. 2 SEQ ID NO: 2 in that lysine at the 3<sup>rd</sup> position in Sequence No. 2 SEQ ID NO: 2 is deleted. Xaa in Sequence Nos. 1 and 2 SEQ ID NOS: 1 and 2 refers to an arbitrary amino acid. Preferable amino acids for Xaa at each position in Sequence No. 2 SEQ ID NO: 2 are shown in the following Table.

Page 7, replace the paragraph beginning at line 2 with the following paragraph:

Examples of the alkaline protease include alkaline proteases having an amino acid sequence shown by Sequence No. 3, 4, or 5 SEQ ID NOS: 4, 6, or 8, or such a sequence in which one or more amino acids are deleted, substituted, or added.

Page 11, replace the paragraph beginning at line 9 with the following paragraph:

Examples of the nucleotide sequence of the alkaline protease of the present invention are shown in Sequence Nos. 3 to 5 SEQ ID NOS: 3, 5 and 7. The nucleotide sequence is not limited to Sequence Nos. 3 to 5 SEQ ID NOS: 3, 5 or 7, and acceptable sequences may include a nucleotide sequence encoding the amino acid sequence shown in Sequence No. 1 or 2 SEQ ID NOS: 1 or 2, and a nucleotide sequence encoding such an amino acid sequence in which one or more amino acids are deleted, substituted, or added. Of these, nucleotide sequences represented by Sequence Nos. 3 to 5 SEQ ID NOS: 3, 5 and 7, or such sequences

in which one or more amino acids are deleted, substituted, or added are preferred. In these cases, deletion, substitution, or addition preferably occurs within the above-described variation of amino acid sequence.

Page 29, replace the paragraph beginning at line 21 with the following paragraph:

The obtained N-terminal sequences are shown in Fig. 7. (SEQ IDS NOS: 9-13).

Pages 29-30, replace the last paragraph with the following paragraph:

20-30 Nucleotides primers (SEQ ID NOS: 14-20 for 5'-terminal of + chain and that of the - chain corresponding to the obtained N-terminal sequences were synthesized (SEQ ID NOS: 9-13). PCR reaction was carried out in a 100- $\mu$ L reaction system by use of a template DNA (100 ng), a primer (20 pmol), and PwoDNA polymerase (product of Boehringer Mannheim). When inverse PCR was performed, Expand<sup>TM</sup> long template PCR system (product of Boehringer Mannheim) was used in a 50- $\mu$ L reaction system. PCR carried out by use of these primers, 9860-N2 SEQ ID NO: 14 and 9860-25k-RV (SEQ ID NO: 17), provided a DNA fragment of 527 bp.

Page 31, replace the last paragraph with the following paragraph:

Inverse PCR was performed by use of primers (1~4 (Fig. 9 (SEQ ID NOS: 21-24) Synthesized from the obtained 527 bp sequence. The KP-9860 chromosome was completely digested by use of restriction enzymes, i.e., *Eco*RI, *Hind*III, *Pst*I, and *Bg*II, and each sample was treated by use of Ligation Kit Ver. 2 (product of Takara) for circularization. Each of the resultant reaction mixtures was served as a template DNA for inverse PCR. PCR reaction (conditions; (94°C-10 seconds, 60°C-30 seconds, 68°C-4 minutes) x 10 cycles; (94°C-10 seconds, 60°C-30 seconds, 68°C-4 minutes + 20 x the number of cycles) x 20 cycles; 68°C-7 minutes; and 4°C-1 minute) was performed by use of the template DNA described above (0.1  $\mu$ g), primers 1 and 4 (10 pmol, respectively), and the Expand Long Plate PCR System. In

addition, PCR (conditions; as described above) was performed by use of the template DNA derived from *Eco*RI digested chromosome (0.1 µg), primers 2 and 3 (10 pmol, respectively), and the Expand Long Plate PCR System. The resultant amplified DNA fragments were purified by use of High Pure PCR Product Purification Kit, and terminals were converted to blunt-ended by use of DNA Blunting Kit (product of Takara). Each of the obtained DNA fragments and *Sma*I digested pUC18 were mixed, and the mixture was treated with Ligation Kit Ver. 2. As described above, *E. coli* JM 109 strain was transformed by the recombinant plasmid, and the obtained recombinant plasmid was served as a template DNA for sequencing. Thus, the nucleotide sequence of the amplified DNA fragments was determined.

Page 33, replace the first paragraph with the following:

(NDVARHIVKADVAQSSYGLY) (SEQ ID NO: 9) which matches the N-terminal sequence of the purified KP9860 protease. Judging from the N-terminal sequence, the mature region of KP9860 protease gene was deduced to be the 1302 bp, encoding 434 amino acid residues (Sequence No. 3 (SEQ ID NO: 4), molecular weight 45310 Da). Upstream of the ORF, there were observed sequences which are deduced to be a promoter region (-35 region: ttgtgt, -10 region: tacgat) and a ribosome-binding site (SD sequence: aggagt). Downstream of the termination codon (taa), there was an inverted repeat having a free energy of -26.2 kcal/mol, which is deduced to be a terminator.

Page 33, replace the second paragraphs with the following

The procedure of Example 5 was repeated, to thereby analyze the entire nucleotide sequence and amino acid sequence of each of the genes of KP-43 protease and KP-1790 protease. The results are shown in Sequence Nos. 4 and 5 SEQ ID NOS: 4 and 5.

Page 48 (Abstract), after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.